Identification of a Major Quantitative Trait Locus Conditioning Resistance to Greenbug Biotype E in Sorghum PI 550610 Using Simple Sequence Repeat Markers

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ABSTRACT Greenbug, *Schizaphis graminum* (Rondani), represents the most important pest insect of sorghum, *Sorghum bicolor* (L.) Moench, in the Great Plains of the United States. Biotype E is the most widespread and dominant type not only in sorghum and wheat, *Triticum aestivum* L., fields, but also on many noncultivated grass species. This study was designed to determine sorghum accession PI 550610 resistance to greenbug biotype E, to map the resistance quantitative trait loci (QTLs) by using an established simple sequence repeat (SSR) linkage map and to identify SSR markers closely linked to the major resistance QTLs. In greenhouse screening tests, seedlings of PI 550610 showed strong resistance to the greenbug at a level similar to resistant accession PI 550607. For QTL mapping, one F_2 population containing 277 progeny and one population containing 233 $F_{2:3}$ families derived from Westland A line × PI 550610 were used to genotype 132 polymorphic SSR markers and to phenotype seedling resistance to greenbug feeding. Phenotypic evaluation of sorghum seedling damage at 7, 12, 17, and 21 d postinfestation in the $F_{2:3}$ families revealed that resistance variation was normally distributed. Single marker analysis indicated 16 SSRs spread over five chromosomes were significant for greenbug resistance. Composite interval and multiple interval mapping procedures indicated that a major QTL resided in the interval of 6.8 cM between SSR markers Xtxp358 and Xtxp289 on SBI-09. The results will be valuable in the development of new greenbug biotype E resistant sorghum cultivars and for the further characterization of major genes by map-based cloning.

KEY WORDS aphid, host resistance, quantitative trait loci, simple sequence repeats, *Schizaphis graminum*

Greenbug, *Schizaphis graminum* (Rondani), represents the most important pest insect of sorghum, *Sorghum bicolor* (L.) Moench, in the Great Plains of the United States. The first extensive damage to sorghum caused by greenbug feeding was recorded during the 1968 crop growing season, and the greenbug isolate was designated as biotype C, because it was virulent to 'Piper' sudangrass, *Sorghum sudanense* (Piper) Stapf (as *S. bicolor* subsp. *drummondii* by de Wet 1978), which was highly resistant to greenbug biotype B (Harvey and Hackerott 1969). In 1979, a new greenbug biotype was collected from a field at Bushland, TX, and the progeny of the collection killed biotype C-resistant wheat, *Triticum aestivum* L., genotype Amigo and its derivatives in greenhouse infestations (Porter et al. 1982). Porter et al. (1982) designated the virulent greenbug as biotype E after extensive comparative tests with greenbug biotype C on small grains and sorghum genotypes. Subsequently, more virulent greenbug biotypes, such as I and K, which can cause serious damage to resistant sorghum cultivars were recognized (Harvey et al. 1991, 1997). It seems that new greenbug biotypes are replacing old biotypes to overcome existing resistant genotypes of sorghum. However, Porter et al. (1997) argued that there was no correlation between the use of resistant sorghum hybrids and the development of new biotypes after careful examination of greenbug–host plants interactions on wheat and sorghum. It is now clear that newly identified greenbug biotypes are actually previously undetected but preadapted opportunists, which may take advantage of genetically uniform hosts and are clonally prolific on transient sorghum or wheat crops (Porter et al. 1997). This suggests that the early identified biotypes, like biotype E, were not replaced by new biotypes; rather, they remain widespread and they will be injurious to sorghum production if sorghum hybrids do not have protection of the respective resistance genes.

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Early genetic studies showed that the inheritance of sorghum resistance to greenbug was simple and partially dominant (Weibel et al. 1972). Olonju Dixon et al. (1990, 1991) indicated sorghum resistance to greenbug biotype E is polygenic, from one to five genes, partially dominant at single locus, and that it can be complementary among resistance loci. Recently, Katsar et al. (2002) studied biotype E resistance quantitative trait loci (QTLs) in three different sorghum sources and found one to three QTLs conferring the resistance in each source. Additional QTL mapping experiments revealed that resistance in disparate sorghums emanated from nonallelic and allelic variation at particular loci for different biotypes C, E, I, and K (Agrama et al. 2002, Katsar et al. 2002, Nagaraj et al. 2005).

Greenbug biotype E, along with biotype I, are the most widespread and dominant biotypes in sorghum growing states in the Great Plains. Porter et al. (1982) observed that biotype E was the predominant biotype in greenbug collections from Bushland, TX, and the greenbug was found ≈75 miles north of Bushland in 1979 and 1980. Kindler et al. (1984) reported that greenbug biotype E was present in most greenbug collections made from wheat and sorghum fields in Kansas, Nebraska, Oklahoma, and northern Texas during 1980–1981. Bowling et al. (1994) also found a wide distribution of biotypes E and I during an extensive field investigation across the Great Plains states of Kansas, Texas, Nebraska, Colorado, and Oklahoma. More recently, the survey of greenbug biotypic diversity in 30 counties of four states—Kansas, Nebraska, Oklahoma, and Texas—by Burd and Porter (2006) demonstrated that biotypes E and I were the only biotypes found in all four states. The two biotypes also enjoyed a wide host plant range from cultivated crops to many noncultivated grass species (Burd and Porter 2006). Therefore, developing new cultivars or hybrids resistant to greenbug biotype E should be one of the major objectives in sorghum breeding programs.

To accomplish this breeding objective, it is necessary to identify and characterize diverse sources of resistance and then to use disparate resistance sources to manage greenbug biotype E. PI 550610, a grain-type sorghum accession, was originally from Syria and introduced into the United States via Russia in 1991 by Andrews et al. (1993). In our preliminary screening test, PI 550610 showed a high level of resistance to biotype E, comparable with resistance in PI 550607, which is a strong resistant accession originating in China (Andrews et al. 1993; Burd and Porter 2006). However, it is not known whether the Syrian sorghum (PI 550610) carries different resistance from PI 550607. The newly developing genotyping methods allow identification of individual resistance genes and more accurate estimates of the number of genes that control the resistance to greenbugs in crop plants.

Simple sequence repeat (SSR) is a widely used marker system because of its high reliability and polymorphism, codominant feature, and easy detection. There have been 352 sorghum genomic SSR primer combinations developed and published by several groups (Brown et al. 1996, Taramino et al. 1997, Dean et al. 1999, Bhattramakki et al. 2000, Kong et al. 2000, Schloss et al. 2002). We used these SSRs to study the genetic components of sorghum resistance to greenbug biotype E. The specific objectives of this study were to examine sorghum accession PI 550610 resistance to greenbug biotype E, to map the resistance QTLs by using an established SSR linkage map, and to identify SSR markers closely linked to the major QTLs of PI 550610. The results will be valuable in the development of new greenbug biotype E resistant sorghum cultivars and for further characterization of major genes by map-based cloning.

Materials and Methods

Sorghum Parental Lines and Populations for Mapping. Originating in Syria, PI 550610 showed a high level of resistance to greenbug biotype I (Andrews et al. 1993). In two screening tests against greenbug biotype E infestation with 20 randomized replications for each test, seedlings of PI 550610 also showed a high level of resistance to greenbug biotype E, whereas Westland A line (NSL 20633) was susceptible to biotype E. Therefore, PI 550610 was selected as the pollen parent to cross with Westland A line to create an F_2 population for genotyping SSR marker polymorphisms, and $F_{2:3}$ families population to phenotype greenbug feeding damage. The cross was made in 2003, and its F_2 population contained 233 progeny. In 2004, $F_{2:3}$ seeds were harvested from each bagged F_2 plant in the field at the USDA–ARS Plant Science Research Laboratory, Stillwater, OK.

Phenotyping Greenbug Biotype E Resistance for Each of the 233 $F_{2:3}$ Families. Two phenotypic screening experiments (test 1 and test 2) were conducted in a greenhouse to collect greenbug damage scores on 12 seedlings of each $F_{2:3}$ family. In each screening experiment, a randomized complete block design with three replicates and two parental lines as controls was used, and damage scores of six individuals were collected for each family, with two seedlings per replicate. In previous studies (Katsar et al. 2002, Agrama et al. 2002), one data point for each seedling was collected when the susceptible control was dying or dead. In this study, greenbug damage scores were collected on each seedling at 7, 12, 17, and 21 d postinfestation (DPI). Using this strategy, we can enhance QTL mapping accuracy by comparing and validating the results of different time points with each other (see Results). Greenhouse experimental procedures based on Starks and Burton (1977) were routinely used in greenbug screening at the USDA–ARS Plant Science Research Laboratory, Stillwater, OK. The greenhouse was maintained at 28 ± 2°C with a photoperiod of 14:10 (L:D) h. For each replicate of the two experiments, five sorghum seeds of each family were randomly sown into a cell of a plastic growing tray filled with a standard soil mix (Scotts-Sierra Horticultural Products Company, Marion, OH), then watered daily to maintain a good moisture level for germination. After about

7 or 8 d, seedlings at the 2–3-leaf stage were thinned to two per cell; the seedlings were healthy and similar in size to the seedlings in other cells. Simultaneously, biotype E greenbugs were prepared by infesting 7-d old 'Schuyler' barley, *Hordeum vulgare* L., seedlings (susceptible to greenbug biotype E) growing in a 10-cm (4-in.) pot with pure Biotype E clones for 8 d. The greenbugs harvested from approximately three barley pots were used to infest one sorghum seedling flat by evenly laying infested barley leaves between two neighboring rows. Greenbug damage to each seedling was scored at the aforementioned days postinfestation (i.e., 7, 12, 17, and 21 DPI) by two experienced technicians, independently. A scale system of one to six was used, with one representing <20% leaf damage to six being dead with equal intervals to the scale.

SSR Genotyping and Data Acquisition. DNA samples for each of the F_2 population plants and the two parents were isolated from young leaf tissue followed the cetyltrimethylammonium bromide procedure developed by Doyle and Doyle (1990). Three hundred fifty two (352) genomic SSR primer combination sequences reported by Brown et al. (1996), Taramino et al. (1997), Dean et al. (1999), Bhattramakki et al. (2000), Kong et al. (2000), and Schloss et al. (2002) were used in the study. First, the genomic SSR markers were screened for polymorphism by using both parents and two randomly selected F_2 individuals. Then, polymorphic SSRs were chosen for genotyping all F_2 progeny and parents to generate marker data by using fluorescence-labeled polymerase chain reaction (PCR) reactions and the LI-COR gel electrophoresis technique (DNA analyzer 4300, LI-COR, Lincoln, NE). SSR PCR reactions were conducted in 96-well PCR plates (Simport Plastics, Beloeil, QC, Canada) in a PTC-220 Thermal Cycler (MJ Research, Watertown, MA). The forward primer of each SSR primer pair was tailed with a M13 forward primer sequence (5′-CAC GACGTTGTAAAACGACG-3′) at the 5′ end during primer synthesis by Invitrogen (Carlsbad, CA). PCR chemical recipe, thermal conditions, and cycles followed the routine procedure outlined in Wu and Huang (2007). PCR products of one plate labeled with 700-nm fluorescent dye and of another plate labeled with 800-nm dye were pooled, mixed, and then 5 μl of LI-COR loading buffer was added. Then, 0.5–0.8 μl of each PCR sample was loaded into each well of a 6.5% KB^{plus} gel (LI-COR) in 1× Tris borate-EDTA buffer and run at a constant 1,500 V for 1–2.5 h according to SSR band size in a LI-COR 4300 DNA analyzer. SSR PCR bands were read into an Excel (Microsoft, Redmond, WA) binary data sheet according to MAPMAKER/EXP 3.0 manual (Lincoln et al. 1992).

Data Analysis. Mean greenbug damage scores and associated standard deviations (SD) were calculated for all families and both parents over each of the four time points—7, 12, 17, and 21 DPI—by using SAS/MEANS. SAS/CORR and MIXED were used for calculation of correlation coefficients and for analysis of variance (ANOVA) (SAS Institute 2003). In the association analyses between polymorphic SSR markers and greenbug damage phenotypic data, the averaged phenotypic values of the greenbug damage ratings for each family and parental line were used. SAS/GCHART was used to generate histograms of the greenbug damage ratings of the four time points (7, 12, 17, and 21) (SAS Institute 2003). SAS/REG was performed to identify the significant ($P < 0.05$) associations of SSR markers with the seedling damage ratings at various time points. Genetic linkage maps were constructed using MAPMAKER 3.0 (Lander et al. 1987) with a threshold value of logarithm of odd ratio (LOD) ≥ 5.0 and the Kosambi mapping function (Kosambi 1944). The chromosomal identities of linkage groups of SSR markers were assigned following Kim et al. (2005) and Menz et al. (2002). For QTL analyses of the greenbug damage ratings for the four time points, composite interval mapping (CIM) and multiple interval mapping (MIM) were performed using Windows QTL Cartographer 2.5 (Basten et al. 2003, Wang et al. 2005). The threshold of significant QTLs in CIM was determined by a 1,000 permutation test (Basten et al. 2003, Wang et al. 2005). The significant *P* value of 0.05 was used for the model selection in MIM.

Table 1. Averaged damage scores in two greenhouse screening tests of three sorghum lines (PI 550610, Westland A line, and PI 550607) against greenbug biotype E infestation

ID	Seedling damage rating				
	Test 1		Test 2		
	7 DPI	17 DPI	7 DPI	10 DPI	14 DPI
PI 550607			1.7	2.2	2.9
PI 550610	2.5	3.8	1.4	2.2	3.0
Westland A line	5.5	6.0	4.3	5.6	6.0

Results and Discussion

Means of sorghum seedling damage scores after greenbug biotype E infestations for two screening tests are given in Table 1. For test 1, PI 550610 showed significantly higher resistance to greenbug attack than Westland A line ($P < 0.01$). For test 2, the damage scores of PI 550610 over three time points—7, 10, and 14 DPI—were similar ($P > 0.05$) to those of PI 550607, which is a strong resistance genotype to greenbug biotype E, and used as a positive control in this test (Burd and Porter 2006). Westland A line had significantly lower resistance to the greenbug feeding than the two resistant lines ($P < 0.01$) in test 2. The results indicated that the germplasm accession PI 550610 is a new source with strong resistance to greenbug biotype E. The F_2 population and $F_{2:3}$ families derived from a cross of Westland A line by PI 550610 were appropriate to map the resistance QTLs.

Means and associated standard deviations, and differences in resistance to greenbug biotype E infestation among sorghum seedlings of both parents and 233 $F_{2:3}$ families in the mapping population at four time points are given in Table 2. Again, PI 550610 had significantly higher resistance to greenbug biotype E than Westland A line ($P < 0.01$) for all DPI scores.

Table 2. Means and associated standard deviations, and differences in resistance to greenbug biotype E among sorghum seedlings of two parents and 233 $F_{2:3}$ families in the mapping population at four time points of postinfestation

Trait	Westland A line (P_1)	PI550610 (P_2)	P_1-P_2	$F_{2:3}$ families
GD7DPI[a]	2.4 ± 0.7[b]	0.6 ± 0.3	1.8**	1.5 ± 0.8**
GD12DPI	5.3 ± 0.3	2.8 ± 0.3	2.5**	3.8 ± 1.0**
GD17DPI	6.0 ± 0.0	3.8 ± 0.1	2.2**	4.9 ± 0.8**
GD21DPI	6.0 ± 0.0	4.6 ± 0.3	1.4**	5.3 ± 0.5**

**, significance at the probability levels of 0.01 in ANOVA.

[a] GD7DPI stands for greenbug damage showing on sorghum seedlings at the 7 d postinfestation.

[b] Damage rating scales given as 1 through 6, being from <20% leaf area damaged to dead, respectively.

Continuous and approximately normal distribution of variations in resistance to greenbug biotype E were observed among the F2:3 families over the four leaf damage ratings (Fig. 1A–D). As shown in Table 3, phenotypic correlation coefficients of the four greenbug damage ratings were high, ranging from 0.82 to 0.97 and significant ($P < 0.0001$). The positive and strong correlations suggested that genetic control of sorghum resistance to greenbug biotype E feeding damages over time is the same or similar.

Table 3. Phenotypic correlation coefficients and associated probability values among the four damage ratings in 233 $F_{2:3}$ sorghum families after infestation by greenbug biotype E

Trait descriptor	GD12DPI	GD17DPI	GD21DPI
GD7DPI	0.90 <0.0001	0.84 <0.0001	0.82 <0.0001
GD12DPI		0.94 <0.0001	0.93 <0.0001
GD17DPI			0.97 <0.0001

Among the 352 publicly available SSR markers, 118 polymorphic markers were mapped into 16 linkage groups of 10 sorghum chromosomes (Wu and Huang 2007). The mapped SSRs spanned ≈1,000 cM in the Kosambi function. Regression analyses of the sorghum seedling damage rating data at all DPI points with the polymorphic markers indicated 16 SSRs were significantly ($P < 0.05$) associated with the reactions of $F_{2:3}$ sorghum seedlings to greenbug feeding (Table 4). Among the markers linked to the greenbug resistance, 10 are responsive at all four time points after greenbug infestation, whereas other markers were associated with the resistance at one or two time points (Table 4). The determination coefficients (r^2) reflects the proportion of genetic variation of the resistance in the

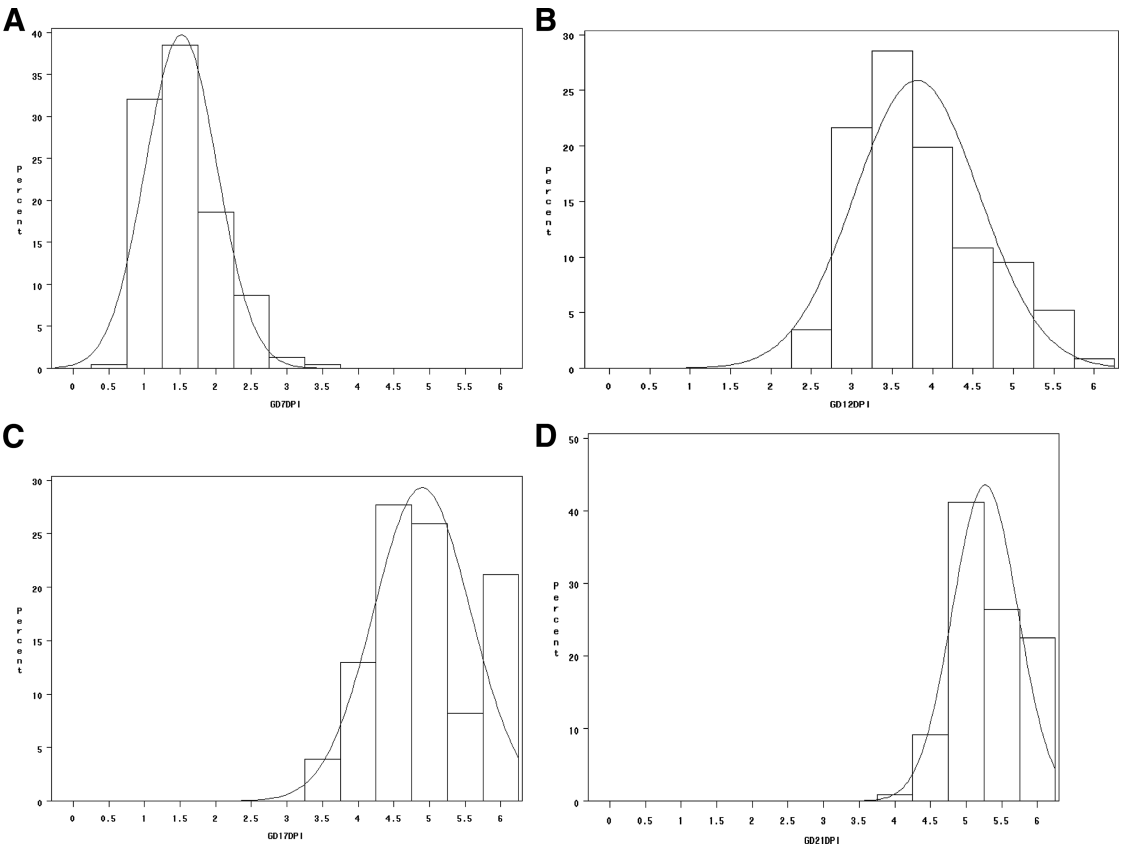

Fig. 1. Frequency distributions (A–D) for four greenbug damage scores at 7, 12, 17, and 21 d postinfestation by greenbug biotype E of 277 $F_{2:3}$ families derived from a cross between Westland A line (susceptible) and PI 550610 (resistant).

Table 4. Determination coefficients (r^2) for 16 SSR markers significantly linked to greenbug damage ratings at either or all of the four time points (GD7DPI, GD12DPI, GD17DPI, and GD21DPI) and associated *P* values in the F_2 mapping population derived from Westland A line × PI 550610

SSR ID[a]	GD7DPI		GD12DPI		GD17DPI		GD21DPI	
	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*
Sb4_15	0.03	0.0091	0.03	0.0150	0.04	0.0033	0.03	0.0099
Sb5_85	0.46	<0.0001	0.55	<0.0001	0.62	<0.0001	0.62	<0.0001
Xtxp10							0.02	0.049
Xtxp67	0.13	<0.0001	0.11	<0.0001	0.14	<0.0001	0.14	<0.0001
Xtxp82	0.02	0.0422	0.02	0.0288				
Xtxp116	0.02	0.0247	0.02	0.0495				
Xtxp131	0.05	0.0010	0.04	0.0022	0.03	0.0055	0.03	0.0116
Xtxp216	0.02	0.0274	0.02	0.0491				
Xtxp230	0.11	<0.0001	0.10	<0.0001	0.13	<0.0001	0.12	<0.0001
Xtxp258	0.17	<0.0001	0.18	<0.0001	0.22	<0.0001	0.21	<0.0001
Xtxp289	0.47	<0.0001	0.58	<0.0001	0.66	<0.0001	0.66	<0.0001
Xtxp320	0.02	0.0360	0.03	0.0157	0.02	0.0348	0.03	0.010
Xtxp358	0.55	<0.0001	0.66	<0.0001	0.70	<0.0001	0.70	<0.0001
Xcup02	0.05	0.0006	0.04	0.0014	0.05	0.0007	0.05	0.0007
Xcup24	0.02	0.0469	0.02	0.0346				
Xcup47	0.02	0.0253						

[a] Xtxp10, Xtxp67, Xtxp82, Xtxp85, Xtxp116, Xtxp131, Xtxp216, Xtxp230, Xtxp258, Xtxp289, Xtxp320, and Xtxp358 were reported by Bhattramakki et al. (2000) and Kong et al. (2000); Sb4_15 and Sb5_85 by Brown et al. (1996); Xcup02, Xcup24 and Xcup47 by Schloss et al. (2002).

mapping population that can be accounted for by the linked marker, which is regulated by the genetic distance of the marker to the QTL, by the magnitude of the QTL itself on the resistance, or both. In this study, r^2 values of the 16 significantly linked markers ranged from 2 to 70%. Six markers Xtxp358, Xtxp289, Sb5_85, Xtxp258, Xtxp67, and Xtxp230 consistently explained >10% of the genetic variation in sorghum resistance to

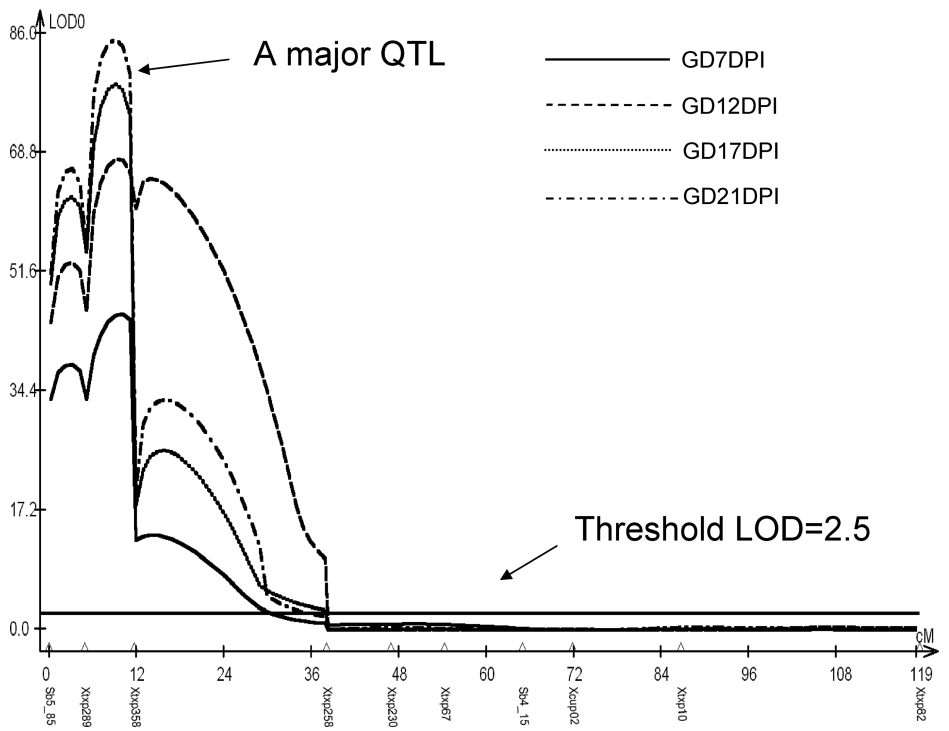

Fig. 2. Linkage map (bottom portion) and CIM QTL LOD profile (top portion) of sorghum chromosome nine (SBI-09) constructed with SSR markers from an F_2 and $F_{2:3}$ population derived from the cross of Westland A line (susceptible) by PI 550610 (resistant) to demonstrate the LOD profiles and a major QTL location for four greenbug damage ratings GD7DPI, GD12DPI, GD17DPI, and GD21DPI. A line with LOD value of 2.5 parallel to the x-axis on the top of the graph indicates the significant threshold of detection of QTL ($P < 0.05$). Genetic distance in centimorgans and SSR marker designations are shown along the linkage map.

greenbug biotype E over all four time points (Table 4). These results suggested that major resistance genes were within short genetic distances to these markers. From the linkage mapping results (Wu and Huang 2007) and previously published data (Tao et al. 1998, Bhattramakki et al. 2000, Kong et al. 2000, Kim et al. 2005), of the 16 significant markers, Xtxp358, Xtxp289, Sb5_85, Xtxp258, Xtxp67, Xtxp230, Xcup02, Sb4_15, Xtxp82, and Xtxp10 were mapped to chromosome 9 (SBI-09); Xtxp116, Xtxp216, and Xcup24 are on chromosome 3 (SBI-03); and Xcup47, Xtxp131, and Xtxp320 on chromosomes 8 (SBI-08), 4 (SBI-04), and 10 (SBI-10), respectively.

QTL analyses including CIM and MIM consistently indicated a major resistance QTL on SBI-09 (CIM graph shown in Fig. 2). The QTL mapping results were in a good agreement with the results of single marker analysis. The QTL was mapped at 9.3 cM from the end of SBI-09 short arm, in the interval of 6.8 cM between two SSR markers Xtxp358 and Xtxp289 by using the standard CIM procedure (Basten et al. 2003). The QTL was 2.4 cM away from Xtxp358 and 4.4 cM from Xtxp289 (Fig. 2). The LOD values of respective peaks were 45.33, 68.00, 78.59, and 85.15 for seedling damage scores of 7, 12, 17, and 21 DPI, respectively (Fig. 2). MIM results indicated that the major QTL accounted for 58.5, 78.1, 83.8, and 84.8% of the observed genetic variation in sorghum resistance to greenbug biotype E measured at 7, 12, 17, and 21 DPI, respectively. The resistance effects were largely attributed to additive gene function by substituting a single copy of a susceptible allele with a resistance allele, the visually rated scores could be reduced by respective 0.54, 0.96, 0.90, and 0.60 U for 7, 12, 17, and 21 DPI, whereas dominant effects were partial, with reduced values from −0.13, −0.26, −0.08, and −0.12 for the ratings of the four time data points. CIM QTL mapping results also indicated a minor QTL in the region between markers Xtxp131 and Xtxp12 on SBI-04, with a LOD value at 7 DPI slightly over the threshold of 2.5.

Recent QTL mapping investigations on sorghum resistance to greenbug feeding damage demonstrate that multiple genomic regions are responsible for greenbug resistance in sorghum (Agrama et al. 2002, Katsar et al. 2002, Nagaraj et al. 2005). Katsar et al. (2002) reported one to three QTLs expressing resistance to greenbug biotype E in each resistant source. The QTLs on linkage group (LG) G (SBI-09) and LG E (SBI-08) accounted for 41 and 49% of the variation in resistance of sorghum source Tx2783 to greenbug biotype E. The QTL linked probes pSB347 and pSB343 were mapped in the same genomic region of the short arm of SBI-09 as that of the major QTL identified in this study according to Feltus et al. (2006), Menz et al. (2002), and Kim et al. (2005). Interestingly, sorghum PI 550607, a strong resistance source also contained a QTL accounting for 26% of the total variation in resistance to greenbug biotype E on the short arm of LG G (SBI-09) (Katsar et al. 2002). The resistance QTL locus was ≈7 cM away from pSB347 (Katsar et al. 2002), and it resided in the interval between Xtxp258 and Xtxp358 on the basis of the map by Feltus et al. (2006). However, the major QTL identified in this study contributed a much higher portion of resistance to greenbug biotype E than previously reported, and it resided in the interval of Xtxp289 and Xtxp358. In a separate study with the same mapping population, we identified four genomic regions (QTLs) that are closely linked the host resistance to greenbug biotype I. These resistance QTLs seemed to have additive and partially dominant effects. Among those greenbug resistance QTLs, two of them reside on the short arm of chromosome 9. This region is also responsible for resistance to biotype C in Tx2737 (Katsar et al. 2002). It seems that the short arm of SBI-09 is rich for greenbug resistance genes.

In this study, no QTL was detected on SBI-08, probably because Westland A line was used as the seed parent. The Westland A line putatively contains the A1 cytoplasmic male sterility system according to Karper (1944) and Stephens and Holland (1954). Recently, the major fertility restorer gene for A1 CMS system was mapped on SBI-08 by Klein et al. (2001, 2005). This mapping population probably reduced our ability to detect resistance QTLs on the chromosomes where male sterile genes reside, such as SBI-08. However, the major QTL detected in this study and the tightly linked SSR markers should be useful for marker-assisted selection and map-based cloning.

Acknowledgments

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